
PHYLOGENETIC RELATIONSHIPS BETWEEN DERIVED APOCYNACEAE S.L. AND WITHIN SECAMONOIDEAE BASED ON CHLOROPLAST SEQUENCES¹

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ABSTRACT

Phylogenetic relationships between derived subfamilies of Apocynaceae s.l. as well as within the subfamily Secamonoideae have been inferred using maximum parsimony and Bayesian analyses. Characters are provided by four chloroplast sequences: *matK*, *trnT-L* and *trnL-F* spacers, and *trnL* intron. Phylogenies from both analyses show a sister group relationship between a clade of derived Apocynoideae included in the subtribe Baisseinae (tribe Apocynae) and another clade including representatives from the Secamonoideae and Asclepiadoideae. Within the Secamonoideae, two genera, *Pervillaea* Decne. and *Secamonopsis* Jum., are strongly supported as monophyletic, whereas the monophyly of the largest genus, *Secamone* R. Br., remains uncertain. Palynological and morphological characters are discussed for the Malagasy *Secamone* in relation to one of the most parsimonious molecular trees.

Key words: Adaptive radiation, Apocynaceae, Madagascar, *matK*, phylogeny, Secamonoideae, *trnL*, *trnL-F*, *trnT-L*.

The Secamonoideae (Endlicher, 1838) are a small subfamily of the former Asclepiadaceae s. str., now included in Apocynaceae s.l. (Endress & Bruyns, 2000). In the treatment of Endress and Bruyns, nine genera were accepted: *Calyptranthera* Klack., *Genianthus* Hook. f., *Goniostemma* Wight, *Pervillaea* Decne., *Rhynchostigma* Benth., *Secamone* R. Br., *Secamonopsis* Jum., *Toxocarpus* Wight & Arn., and *Trichosandra* Decne. *Goniostemma*, however, is of uncertain taxonomic validity (Venter & Verhoeven, 1997), and the monotypic African genus *Rhynchostigma* has recently been put into synonymy under *Secamone* (Klackenberg, 2001). The Secamonoideae have fewer than 200 species, restricted mostly to the Old World tropics. *Secamone* is the largest genus, with more than 90 species that occur mainly in Madagascar and the Mascarene Islands (Klackenberg, 1992a), Africa (Goyder, 1992; Klackenberg, 2001; Bruyns, 2004), Australia, and Asia (Forster & Harold, 1989; Klackenberg, 1992b). *Toxocarpus*, with more than 50 species, occurs mainly in Asia, as does *Genianthus*.

with 16 species (Klackenberg, 1995a). The four other genera, *Pervillaea* (Klackenberg, 1995b, 2005a), *Secamonopsis* (Civeyrel & Klackenberg, 1996), *Calyptranthera* (Klackenberg, 1996a, b, 1997a), and *Trichosandra* (Friedman, 1990), are restricted to Madagascar or to the Mascarene Islands, with fewer than 10 species each. The main center of biodiversity for the subfamily is Madagascar (Klackenberg, 1992a), where about half of the known species and genera occur, followed by Southeast Asia, and then Africa (Klackenberg, 1992b, 1995a, 2001).

Recent studies appraising the phylogeny and systematics of the Apocynaceae s.l. have shown the Secamonoideae to be a monophyletic sister group to the Asclepiadoideae (Sennblad & Bremer, 1996, 2000, 2002; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Fishbein, 2001; Potgieter & Albert, 2001; Lahaye et al., 2005). However, the sister group to the larger clade including Secamonoideae and Asclepiadoideae is still not unambiguously identified. Molecular phylogenies based on the chloroplast gene *matK*

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have shown the Periplocoideae to be the sister group to the Asclepiadoideae–Secamonoideae clade (Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Fishbein, 2001; Lahaye et al., 2005), and the tribe Apocynae (Apocynoideae) to be the sister group to the former Asclepiadaceae (Civeyrel et al., 1998; Civeyrel & Rowe, 2001). This has not been the case in studies based on the chloroplast gene *rbcL* (Sennblad & Bremer, 2000, 2002) and the intron *trnL* combined with the spacer *trnL-F* (Potgieter & Albert, 2001), in which the tribe Apocynae appeared polyphyletic and one of its members, *Bai-sea* A. DC., was sister to a group including Secamonoideae and Asclepiadoideae, though only weakly supported.

The most inclusive phylogeny of the Secamonoideae to date (Lahaye et al., 2005) was based on the two chloroplastic sequences *matK* and *trnT-L* (2543 characters, 9% parsimony informative) and included 26 taxa from three genera related to the Secamonoideae (around 15% of the total species in the subfamily) from Madagascar and the Mascarene Islands. Although this study included a large sampling combined with a high number of characters, it did not resolve the internal relationships between genera. *Pervillaea* and *Secamonopsis* were strongly supported as monophyletic, but a polytomy between these two genera, a second clade consisting of *Secamone oleaefolia* Decne. from coastal eastern Madagascar and *S. volubilis* (Lam.) Marais from La Réunion, and a third clade of other Malagasy *Secamone*, made the monophyly of this last genus uncertain. The study further indicated that several clades within Malagasy *Secamone* were congruent with morphological (Klackenberg, 1992a) or palynological (Civeyrel, 1994) observations.

The Secamonoideae are the central focus of this paper. The principal aims are to clarify relationships between the Secamonoideae and derived Apocynaceae s.l. and to increase the resolution of the internal phylogenetic relationships within the subfamily. We combined previously published sequences from the two chloroplast regions *matK* and *trnT-L* (Endress et al., 1996; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2005) with two other chloroplast regions, the *trnL* intron and the *trnL-F* spacer. For each new region, six previously published sequences (Potgieter & Albert, 2001; Liede & Täuber, 2002) have been used in the present study. Three new taxa forming the subtribe Baisseinae (Apocynae) have been added to the 45 taxa included in the analysis of Lahaye et al. (2005).

The *trnL* intron and the *trnL-F* spacer are non-coding regions of chloroplast DNA (cpDNA) for which universal primers exist (Taberlet et al., 1991). Those plastid regions have proved useful for resolving

infrafamilial relationships among different groups of plants and have previously been used to resolve relationships within the Apocynaceae s.l. (Potgieter & Albert, 2001) and the Asclepiadoideae (Liede & Täuber, 2000, 2002; Liede, 2001; Liede et al., 2002, 2005; Meve & Liede, 2002; Rapini et al., 2003; Yamashiro et al., 2004). It is thought useful to combine these plastid regions, because the relatively low variation of the *trnL* intron (Taberlet et al., 1991) is supposed to structure the topology, whereas the relatively high variation of the *trnL-F* (Taberlet et al., 1991) spacer is supposed to resolve interspecific relationships.

MATERIAL AND METHODS

INGROUP AND OUTGROUP SAMPLING

Taxa were sampled from three genera of Secamonoideae in Madagascar: *Pervillaea*, *Secamonopsis*, and *Secamone*. Appendix 1 provides information on the taxa, voucher specimens, and GenBank accession numbers for all sequences. The three subfamilies Secamonoideae, Asclepiadoideae, and Periplocoideae, together with three taxa of derived Apocynoideae, constitute the ingroup. The outgroup taxa were selected from Apocynoideae and Rauvolfioideae. To the *matK* and *trnT-L* sequences published previously (Endress et al., 1996; Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2005), we added three new sequences of *matK* and 42 new sequences each of *trnL-F* and *trnL* (Appendix 1).

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

DNA extraction, amplification, and sequencing for sequences previously published follow Lahaye et al. (2005); only new protocols used for this study will be described here. Total DNA used for the new sequences was extracted from 20–40 mg of herbarium material by beating the material in tubes containing plastic beads, and then treating samples with the Qiagen DNAeasy plant kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) amplifications were conducted using 2 µl of total DNA.

The chloroplast gene *matK* was PCR amplified in two crossing parts using the primer pairs *matK* 8F and *matK* 829 R, and *matK* 681F and *matK* 1628R (Endress et al., 1996; Civeyrel et al., 1998). The *trnL* intron and *trnL-F* spacer of the chloroplast genome was PCR amplified using the primer pairs c and d and e and f, respectively, designed by Taberlet et al. (1991). These PCR amplifications were carried out using the kit ready-to-go PCR beads (Amersham Biosciences, U.S.A.). These beads containing a premix with 2.5 units of DNA polymerase were reconstituted

to 25 μ L final volume in which the concentration of each dNTP is 200 μ M, with 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, and 1.5 mM $MgCl_2$. To amplify the inferred sequences, the same protocol for thermal reactions was carried out, as follows: (1) one initial cycle of 5 min. at 95°C to ensure denaturation of the double-stranded template DNA; (2) three cycle steps repeated 40 to 44 times: denaturation at 95°C for 15 sec., annealing at 50°C for 1 min., and extension at 72°C for 1 min. 30 sec.; and (3) a final extension step at 72°C for 5 min. to complete unfinished DNA strands. The PCR reactions were purified using the QIAquick spin columns following the manufacturer's protocols (Qiagen, Helden, Germany) and sequenced at the Molecular Systematic Laboratory in Stockholm (Swedish Museum of Natural History) using the Thermo Sequenase Sequencing kit from Amersham Pharmacia Biotech. Sequencing reactions were electrophoresed on 6% Long Ranger gels on a Pharmacia ALF-express automated sequencer. Both strands of DNA were sequenced using the same primers as those used for amplification.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

New data files for *matK*, *trnL*, and *trnL-F* sequences were assembled and edited using the Staden Package (Staden, 1996). Ambiguous positions were coded using appropriate IUPAC (International Union of Pure and Applied Chemistry) ambiguity symbols so as to maximize retention of information. We eliminated the first 50 bases at the 5' and 3' ends of *matK*, the 5' end of *trnL*, and the 3' end of *trnL-F*, because the nucleotide sequences were not completely resolved near the primers and were not confirmed by overlapping sequences. Sequences were initially aligned using ClustalX version 1.8 (Thompson et al., 1997), and the alignment was improved visually. Indels found in the alignment of *matK*, *trnT-L*, *trnL*, and *trnL-F* were included in the analysis but not coded. Their phylogenetic value will be discussed for the Secamonoideae.

Phylogenetic analyses were performed using maximum parsimony (MP), and for comparison, a Bayesian method of phylogenetic inference (Mr. Bayes, ver. 3.1; Huelsenbeck & Ronquist, 2001) was employed. Parsimony analysis was conducted assuming unordered character states. Gaps were treated as missing data. Parsimony analyses were completed using the software package PAUP* version 4.0b10 (Swofford, 2002) with a heuristic search strategy. Each data matrix was analyzed using 1000 replicates of random taxon addition holding 10 trees per replicate to find islands of equally parsimonious trees with tree bisection-reconnection (TBR) on. Complete swapping

was performed on all trees accumulated in the replicates (which should have found all trees at that length) with a tree limit of 40,000 and MULTREES on. Rounds of successive approximation weighting (hereafter SW; Farris, 1969; see also Civeyrel et al., 1998) were added, with characters re-weighted according to their rescaled consistency index (RC), based on best fit characters. Three re-weighting steps were repeated until the tree length remained unchanged over two successive iterations. To evaluate clade support, bootstrap (BS) analyses (Felsenstein, 1985) were performed using a full heuristic search with 1000 replicates, retaining BS frequencies from 1% to 100%, which were compatible with the 50% majority rule consensus tree, simple sequence addition, sampling characters with equal probability but applying weight (from SW), TBR, MULTREES on, and a tree limit of 1000. Bootstrap percentages (BP) are referred to as high (85%–100%), moderate (75%–81%), or low (50%–74%). All illustrated trees use ACCTRAN optimization. The base weight of 1000 applied in SW was removed for tree presentation.

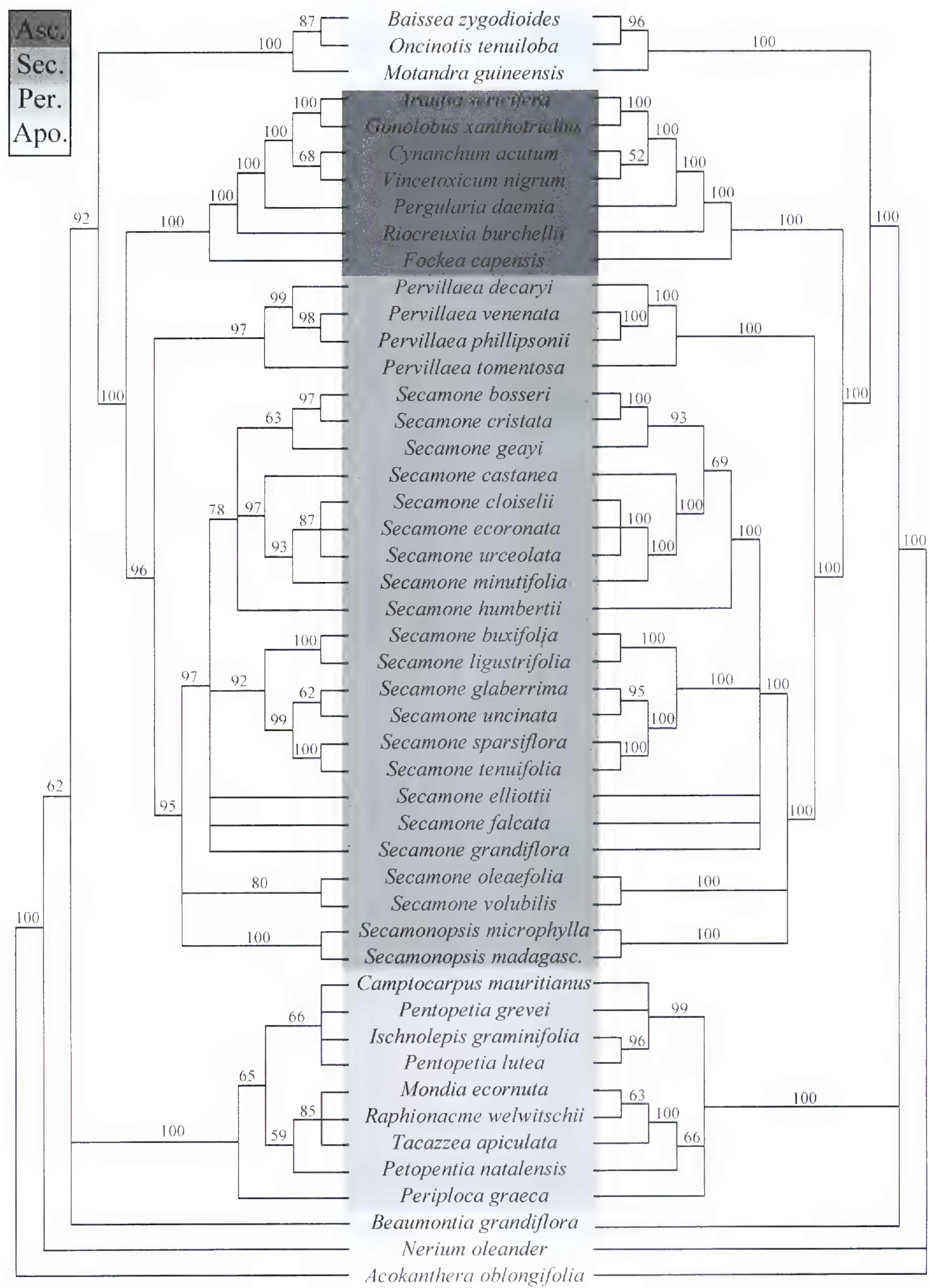
The incongruence length difference (ILD; Farris et al., 1994) test was employed to detect incongruence among the data sets using partition homogeneity test in PAUP*. The partition *matK* versus *trnT-L* versus *trnL* versus *trnL-F* homogeneity test was performed using 750 replicates on parsimony informative characters (Cunningham, 1997), TBR branch-swapping, 100 replicates of random addition sequence holding one tree per replicate, and MULTREES on (MAXTREE = 1000, and other default settings).

The Bayesian analysis was conducted using a general time reversible (GTR + I + G) model determined to be the best fit model for our data sets using Modeltest ver. 3.7 (Posada & Crandall, 1998), with gamma distributed rate variation across sites and an initial estimate of equal base frequencies (Jukes & Cantor, 1969). Two Markov Monte Carlo chains were run simultaneously starting from random trees. The analysis was run for 2,000,000 generations, sampling a tree every 500 generations with a burn-in value (number of trees ignored) of 1000 (25% of total trees saved). The 3001 remaining trees retained after reaching the stationary phase (after ca. 30,000 generations) were used to build a majority rule consensus tree with PAUP*, which is shown as a cladogram with posterior probability values in Figure 1.

RESULTS

DNA MATRIX FEATURES AND SEQUENCE DIVERGENCE

Length variation of the inferred sequences ranged from 590 (*Ischnolepis graminifolia* (Costantin &



Parsimony bootstrap

Bayesian majority rule

Figure 1. Comparison of parsimony and Bayesian phylogenetic trees inferred from the analysis of the combined matrix. The parsimony tree is the strict consensus tree of the 14,400 most parsimonious trees (length = 1153, CI = 0.94) obtained from maximum parsimony followed by tree iterations of successive weighting. Numbers above branches in the parsimony and Bayesian trees are bootstrap values and Bayesian posterior probabilities, respectively. Note that the Bayesian and parsimony topologies are highly congruent. Abbreviations: Apo. = Apocynoideae, Asc. = Asclepiadoideae, Per. = Periplocoideae, Sec. = Secamonoideae.

Table 1. Characteristics of the *matK*, *trnT-L*, *trnL*, *trnL-F*, and combined sequences* constituting the matrices.

	<i>matK</i>	<i>trnT-L</i>	<i>trnL</i>	<i>trnL-F</i>	Combined matrix
Length range (bp)	590–1505	262–751	474–509	358–454	2153–3414
Aligned length (bp)	1547	989	537	484	3557
No. of parsimony-uninformative sites	262	121	49	66	498
No. of parsimony-informative sites	162	76	34	37	309
No. of constant sites	1123	792	454	381	2750
% parsimony-informative sites	10.47	7.68	6.33	7.64	8.69
No. of indels (total)	17	76	22	31	146
No. of autapomorphic indels	10	45	12	21	88
Ingroup sequence divergence (%)	0.06–8	0–8	0–6.7	0–8.6	0.03–7.5
Ingroup/outgroup sequence divergence (%)	3.2–8.5	1.4–8.3	1.2–6.8	1.2–7.9	2.4–7.9
G+C content range (%)	31.8–35.4	19.4–26.3	35.8–38.1	34.2–38.2	31.8–35.6
Transitions (total)	24,446	7374	5202	5063	42,085
Transversions (total)	24,259	10,312	4077	6761	45,409
Transitions/transversions	1.01	0.72	1.28	0.75	0.93

* Sequence divergence was estimated using uncorrected pairwise distances.

Gallaud) Klack., incomplete sequence) to 1505 bp for *matK*, 262 (*Secamone glaberrima* K. Schum., incomplete sequence) to 751 bp for *trnT-L*, 474 to 509 bp for *trnL*, 358 (*S. castanea* Klack., incomplete sequence) to 454 bp for *trnL-F*, and 2153 to 3414 bp for the combined matrix (Table 1). Aligned sequence lengths for these same regions were 1547, 989, 537, 484, and 3557 bp, respectively. Alignments showed 17 indels in the *matK* matrix, 76 indels in *trnT-L*, 22 in *trnL*, 31 in *trnL-F*, and 146 in the combined matrix. The G+C content ranged from 31.8% to 35.4% in *matK*, 19.4% to 26.3% in *trnT-L*, 35.8% to 38.1% in *trnL*, 34.2% to 38.2% in *trnL-F*, and 31.8% to 35.6% in the combined matrix. Sequence divergence between ingroup and outgroup taxa ranged from 3.2% to 8.5% in *matK*, 1.4% to 8.3% in *trnT-L*, 1.2% to 6.8% in *trnL*, 1.2% to 7.9% in *trnL-F*, and 2.4% to 7.9% in the combined matrix. Sequence divergence among ingroup taxa ranged from 0.06% to 8% in *matK*, 0% to 8% in *trnT-L*, 0% to 6.7% in *trnL*, 0% to 8.6% in *trnL-F*, and 0.03% to 7.5% in the combined matrix.

PHYLOGENETIC RESULTS

The number of parsimony-informative sites (excluding indels) detected in the sequences inferred was 162 in the *matK* region (10.47% of the total number of sites), 76 in *trnT-L* (7.6%), 34 in *trnL* (6.34%), 37 in *trnL-F* (7.64%), and 309 in the combined matrix (8.69%) (Table 1). The result of the ILD test was significant ($P = 0.02$), indicating that we could reject the null hypothesis of data set homogeneity ($P < 0.05$) (Farris et al., 1994). The low number of informative sites within both the *trnL* and *trnL-F* matrices relative to the number of taxa creates considerable polytomies

in the reconstruction and heterogeneity between the phylogenetic information given by the data sets. Moreover, there is no apparent reason to suspect incongruence among different regions of a uniparentally inherited and non-recombining genome. Thus, the following results and comments will be based only on the phylogenetic analyses of the combined matrix.

The successive weighting analyses followed the first step of maximum parsimony of the combined matrix with all characters included. This reduced the number of trees from 40,000 to 14,400 (1153 steps without weights and 797,692 weights included) after three iterations and increased the consistency index (CI) from 0.80 to 0.94 (0.62 to 0.84 uninformative characters excluded), the retention index (RI) from 0.83 to 0.95, and the RC from 0.67 to 0.90. Topologies of the two strict consensus trees resulting from complete swapping and successive weighting, respectively, are almost identical. The relationships within the Periplocoideae are better resolved by SW analysis than complete swapping, with three more branches resolved. On the consensus tree obtained after SW (Fig. 1), the monophyly of each of the three subfamilies of the Asclepiadaceae s. str. is strongly supported with a BS of 100% for the Periplocoideae, 100% for the Asclepiadoideae, and 96% for the Secamonoideae. Furthermore, representatives from the Secamonoideae form, together with their sister group Asclepiadoideae, a highly supported clade (BS = 100%) (Fig. 1). A group of Apocynoideae consisting of *Baissea zygodiodes* Stapf, *Oncinotis tenuiloba* Stapf, and *Motandra guineensis* (Thonn.) A. DC. (BS = 100%) is monophyletic and sister to the Asclepiadoideae–Secamonoideae clade (BS = 92%). Because we do not have *trnT-L* sequences for *Baissea*, *Oncinotis* Benth., and *Motandra* A. DC. (Appendix 1),

we performed a supplementary analysis based on *matK*, *trnL*, and *trnL-F* to judge if the absence of those sequences for *trnT-L* might cause a bias into our results. This supplementary analysis supported the *Baissea*–*Oncinotis*–*Motandra* clade with a BS of 88%.

Within the Secamonoideae, 13 groups are strongly supported with BS above 90% (Fig. 1). The genus *Pervillaea* with all Malagasy species included (*P. decaryi* (Choux) Klack., *P. venenata* (Baill.) Klack., *P. phillipsonii* Klack., and *P. tomentosa* Decne.) is strongly supported (BS = 97%). A fifth species of *Pervillaea* has recently been described from Mauritius (Klackenberg, 2005a). However, only 150-year-old material is known for *P. brevirostris* Klack., and it has not yet been possible to include this taxon in the analysis. The bispecific genus *Secamonopsis* is also well supported (BS = 100%), but its relationship with other genera is not resolved. Within the genus *Secamone*, several clades are well supported. This is the case for the clade including *S. cristata* Jum. & H. Perrier and *S. bosseri* Klack. (see also Fig. 2, clade 1, BS = 97%); a clade with *S. castanea*, *S. cloiselii* Choux, *S. ecoronata* Klack., *S. minutifolia* Choux, and *S. urceolata* Klack. (Fig. 2, clade 2, BS = 97%), in which *S. cloiselii*, *S. ecoronata*, *S. minutifolia*, and *S. urceolata* form a strongly supported group (BS = 93%); and a clade composed of *S. ligustrifolia* Decne. and *S. buxifolia* Decne. (Fig. 2, clade 3, BS = 100%) that lies sister to a clade including *S. glaberrima*, *S. uncinata* Choux, *S. tenuifolia* Decne., and *S. sparsiflora* Klack. (Fig. 2, clade 4, BS = 99%) in which *S. tenuifolia* and *S. sparsiflora* form a strongly supported group (BS = 100%).

The Bayesian consensus tree is highly concordant with the consensus tree based on SW (Fig. 1). It shows two more resolved nodes (one within the Periplocoideae and one within the Secamonoideae) than the parsimony consensus tree, but these nodes are not well supported. Support values (posterior probabilities, hereafter PP) for clades in the Bayesian tree, however, are generally higher than those based on parsimony. The number of clades highly supported (PP > 95%) within the Secamonoideae is 17 in the Bayesian phylogeny instead of 14 (BS > 85%) in the parsimony phylogeny (Fig. 1). Like the parsimony phylogeny, the Bayesian tree also identifies two groups of species within the principal genus *Secamone* (PP = 100%): the first including *S. oleaefolia* and *S. volubilis* (BS = 80%; PP = 100%), and the second including the other Malagasy *Secamone* (BS = 97%; PP = 100%). These two groups form a polytomy with *Secamonopsis* in both analyses. Groups of *Secamone* identified in the parsimony tree are also present in the Bayesian tree with higher PP.

DISCUSSION

MONOPHYLY OF THE SECAMONOIDEAE AND THEIR RELATIONSHIPS WITHIN APOCYNACEAE

Both parsimony and Bayesian analyses of the combined four chloroplastic regions *matK*, *trnT-L*, *trnL*, and *trnL-F* support the monophyly of each of the three subfamilies of the former Asclepiadaceae (Asclepiadoideae, Periplocoideae, and Secamonoideae) with the Secamonoideae as sister to the Asclepiadoideae (Fig. 1). *Baissea zygodioides*, *Oncinotis tenuiloba*, and *Motandra guineensis* (Apocynoideae) form a monophyletic sister group to the clade constituted of Asclepiadoideae and Secamonoideae, which renders the former Asclepiadaceae s.l. (i.e., with Periplocoideae included) non-monophyletic. The monophyly of the Asclepiadaceae s. str. has been questioned by addition of derived Apocynoideae such as *Prestonia* R. Br., *Baissea*, and *Motandra* in phylogenetic studies (Sennblad & Bremer, 2000, 2002; Potgieter & Albert, 2001). Although *Baissea* and *Motandra* do not show clear morphological similarities with the Asclepiadaceae, these phylogenies showed *Baissea* to be placed within the former Asclepiadaceae but with a low support value. To date, the phylogenetic studies of derived Apocynaceae s.l. have agreed on the position of Secamonoideae as sister group to the Asclepiadoideae. Our study suggests that a group of derived Apocynoideae composed of *Baissea*, *Motandra*, and *Oncinotis* comes out as sister group to the Asclepiadoideae–Secamonoideae clade. This result is consistent with that of Livshultz et al. (2007) and does not contradict the position of *Baissea* in the analyses of Potgieter and Albert (2001) and Sennblad and Bremer (2002). *Baissea*, *Motandra*, and *Oncinotis* form the subtribe Baisseinae published by De Kruif in 1983 and recognized on a tribal level by Endress (M. Endress, pers. comm.). The Baisseinae could provide a more accurate outgroup for further studies on the phylogeny of Secamonoideae.

RELATIONSHIPS WITHIN SECAMONOIDEAE

Within the Secamonoideae the monophylies of the two Malagasy genera *Pervillaea* and *Secamonopsis* are strongly supported, with BS of 97% and 100%, respectively, and PP of 100% for both genera (Fig. 1). The addition of *trnL* and *trnL-F* sequences increased support of the *Pervillaea* clade from 73% (Lahaye et al., 2005) to 97% but did not resolve the relationships between *Secamone* and *Secamonopsis*. In our study the genus *Pervillaea* is sister to a clade of *Secamone* and *Secamonopsis* species. This result is congruent with a preliminary morphological phylogeny of the Seca-

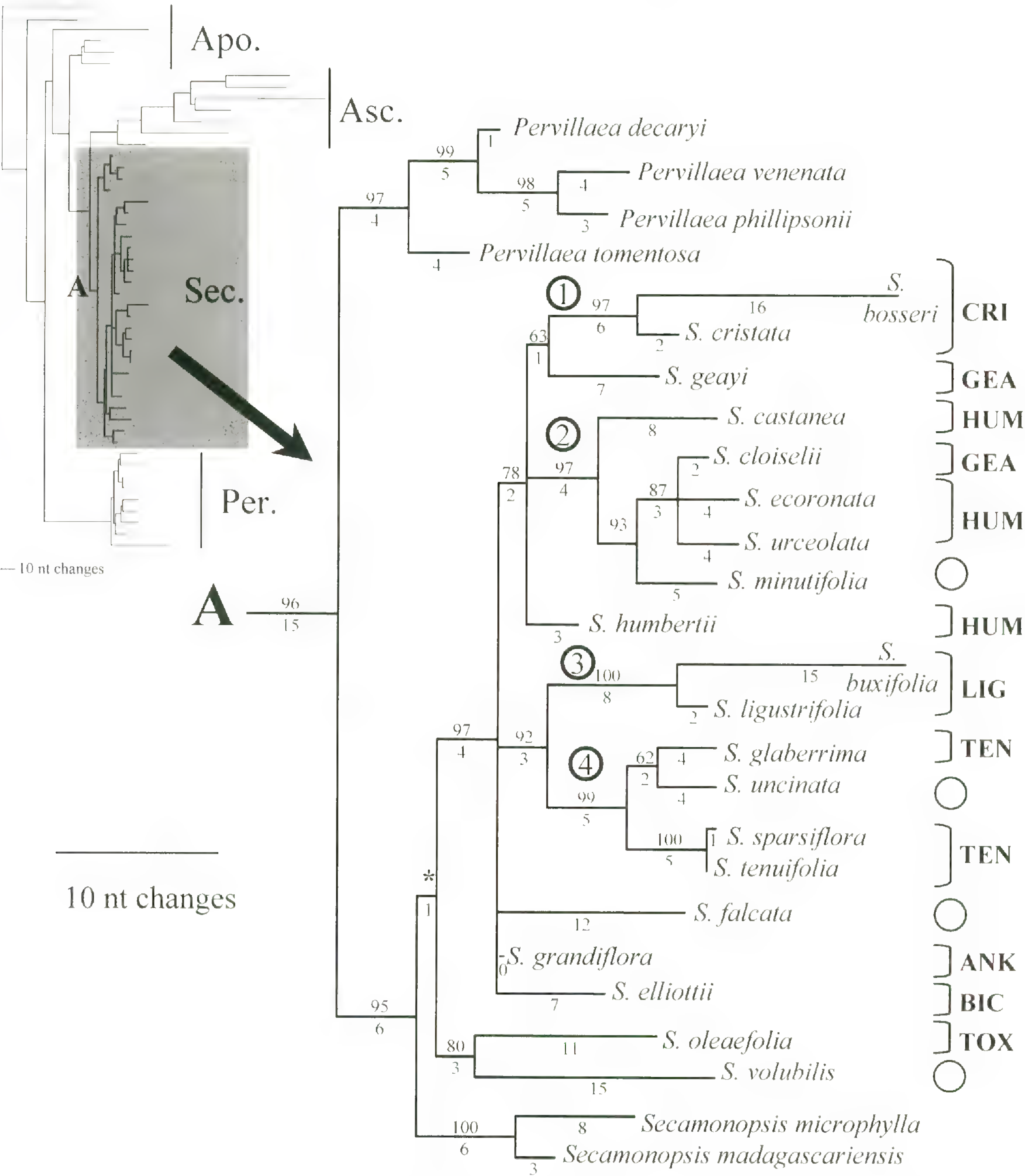


Figure 2. One of the most parsimonious trees represented as phylogram inferred from parsimony analysis (maximum parsimony + successive weighting). Bootstrap values are above branches and branch lengths below; branch scale below at the left represents 10 nucleotide (nt) changes. Morphological groups of Klackenberg (1992a) are localized on the tree. Asterisk on branch represents bootstrap value less than 50%. Abbreviations: Apo. = Apocynoideae, Asc. = Aselepiadoideae, Per. = Periplocoideae, ANK = *S. ankarensis* group, BIC = *S. bicolor* group, CRI = *S. cristata* group, GEA = *S. geayi* group, HUM = *S. humbertii* group, LIG = *S. ligustrifolia* group, TEN = *S. tenuifolia* group, TOX = *S. toxocarpoides* group, O = species not allocated to morphological group.

monoideae (Klackenberg, 1996b), which places *Pervillaea* and *Calyptanthus* as a sister group to a clade including *Genianthus*, *Toxocarpus*, *Secamone*, and *Secamonopsis*. The genus *Pervillaea* is distributed in the northern, western, and southern parts of Madagascar but is not known from the eastern part or from the central high plateau of Madagascar

(Klackenberg, 1996b). *Pervillaea tomentosa* appears to be found in both the humid and dry deciduous forests of western Madagascar and has a larger ecological amplitude compared to the three other species, which are mainly restricted to the drier evergreen southern zones. The morphology-based phylogeny of *Pervillaea* (Klackenberg, 1996b) showed

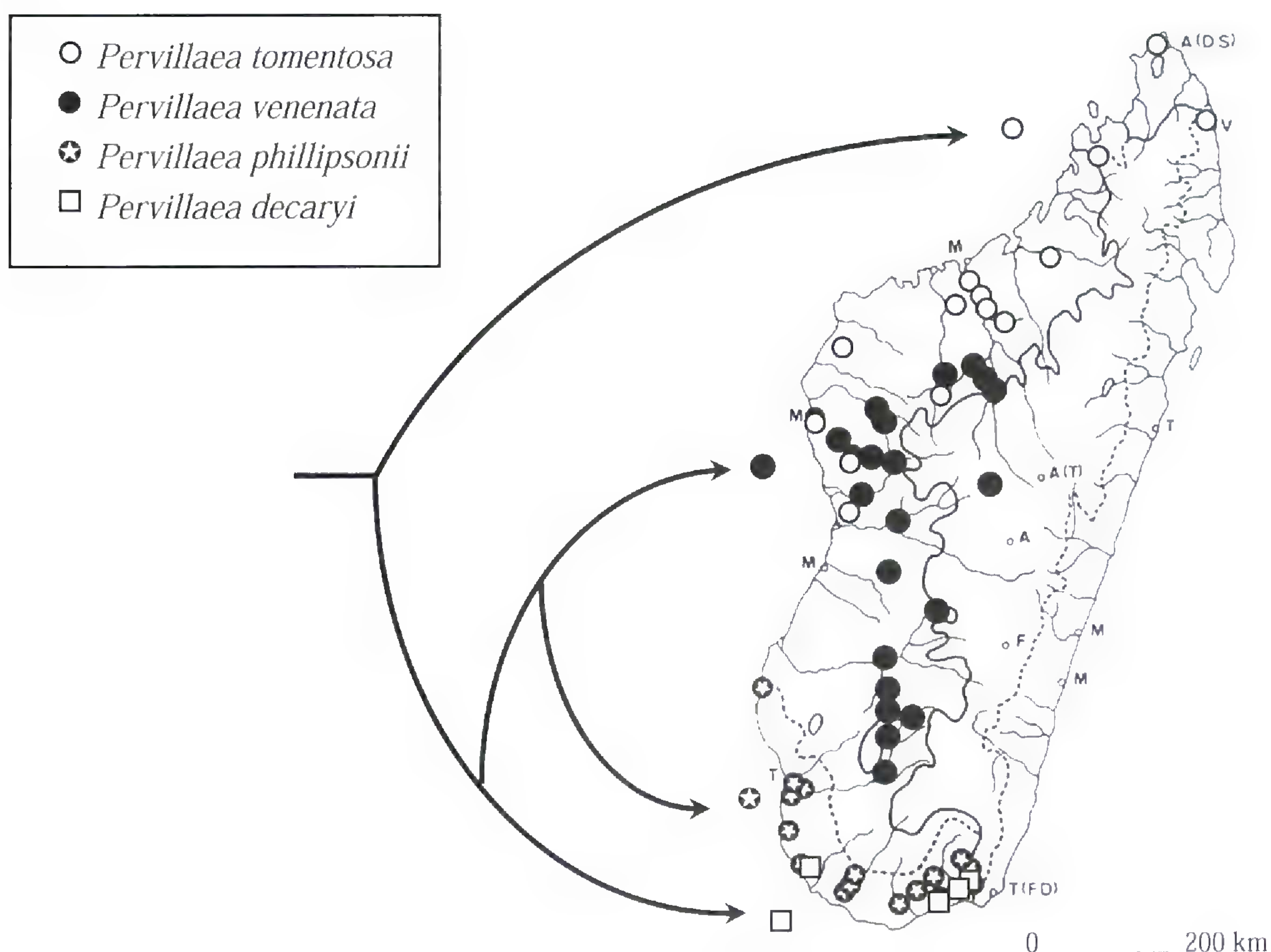


Figure 3. Schematic representation of vicariance pattern occurring within *Pervillaea* in Madagascar. Species distribution from Klackenberg (1996b).

a pattern of parallel vicariance events between western and southern phytogeographical domains in Madagascar, whereas our molecular tree suggests subsequent vicariant events (Fig. 3).

The bispecific genus *Secamonopsis* is monophyletic, but its relationship with the main genus *Secamone* remains unclear (Fig. 1). This result follows previous molecular (Civeyrel et al., 1998; Civeyrel & Rowe, 2001; Lahaye et al., 2005) and morphological (Civeyrel & Rowe, 2001; Klackenberg, 1996b) studies. The position of *Secamonopsis* in a polytomy with one clade including *S. oleaefolia* and *S. volubilis* and a second clade consisting of the remaining *Secamone* exemplars, as shown in both the parsimony and Bayesian trees of the present study (Fig. 1), as well as in earlier studies (Civeyrel & Rowe, 2001; Lahaye et al., 2005), makes the monophyly of *Secamone* uncertain. *Secamone oleaefolia* and *S. volubilis* form a well-supported clade, but these two species do not have clear morphological or biogeographical similarities (Klackenberg, 1992a). The first is distributed on the central plateau of Madagascar, and the second is found on the island of La Réunion. *Secamone volubilis* does not share closer similarities with other *Secamone*, except with *S. salicifolia* Klack. from Mauritius, which has the same flower structure but differs in leaf shape and leaf

pubescence. In contrast, *S. oleaefolia* is, on morphological grounds, thought to be related to *S. obovata* Decne. from the Malagasy eastern coastal forest and to the four species of the *S. toxocarpoides* Choux group from the Malagasy central plateau and northeastern part of the island (Klackenberg, 1992a, 2000a). They all share the characters of having long and narrow corolla lobes, long style-heads, thin-walled linear follicles, and subcoriaceous glossy leaves when dry. Species belonging to this group, including also the Socotran *S. socotrana* Balf. f., are morphologically similar to the genus *Toxocarpus* by, for example, their large flowers (usually ca. 1 cm long) and long style-head (2–3 mm long) (Klackenberg, 1992a). The addition of two new chloroplast sequences in this study did not resolve the polytomy observed between *Secamonopsis*, the *S. volubilis*–*S. oleaefolia* clade, and a clade of the remaining Malagasy species of *Secamone*.

RELATIONSHIPS WITHIN MALAGASY SECAMONE

Morphological (Klackenberg, 1992a) and palynological studies on the genus *Secamone* (Civeyrel, 1994; Civeyrel & Rowe, 2001) have identified characters describing groups of species within the genus. Based on both floral and vegetative characters,

Klackenberg (1992a) discussed several morphological groups within Malagasy *Secamone*, of which eight are applicable to the taxa analyzed in the present article and are mapped on Figure 2: *S. ankarensis* (Jum. & H. Perrier) Klack. group, *S. bicolor* Decne. group, *S. cristata* group, *S. geayi* Costantin & Gallaud group, *S. humbertii* Choux group, *S. ligustrifolia* group, *S. tenuifolia* group, and *S. toxocarpoides* group. Groups of *Secamone* have also been described from palynological studies, which identified six types of pollinaria (Civeyrel, 1994). The present molecular phylogeny allows us to evaluate the phylogenetic value of the morphological and palynological characters in regard to four representative clades including one or two morphological groups (Fig. 2).

Clade 1. *Secamone bosseri* and *S. cristata* of this clade (Fig. 2), representing the *S. cristata* morphological group of three species and three subspecies, are characterized by having small flowers (only 2–2.5 mm long) with long corolla tubes (tubes distinctly longer than the lobes) and usually dense inflorescences (Klackenberg, 1992a). They constitute a strongly supported clade (PP = 100%, Fig. 1). The Bayesian analysis shows that *S. geayi* forms a well-supported clade (PP = 93%) with these two species, but this is not the case after successive weighting (BS = 63%). *Secamone geayi*, *S. cristata*, and *S. bosseri* share the same type of compact pollinarium (Civeyrel, 1994). *Secamone geayi* and *S. bosseri* are restricted to the dry southwestern part of Madagascar, whereas the range of the *S. cristata* subspecies also extends as far as the northwest of the island. The three species are sympatric in southwestern Madagascar.

Clade 2. This clade (BS = 97%) is formed by four species from two different morphological groups and one species that does not fit into any group (Klackenberg, 1992a): *Secamone castanea*, *S. ecoronata*, *S. urceolata* (*S. humbertii* group), *S. cloiselii* (*S. geayi* group), and *S. minutifolia* (unidentified group). The *S. humbertii* group of Klackenberg (1992a, 1997b) consists of 10 species, of which four are included in the present study, viz., *S. castanea*, *S. ecoronata*, *S. urceolata*, as well as *S. humbertii*, which is placed in a polytomy just outside clade 2. This species group is characterized by having pouch-like structures on the stamens, but this character is absent in *S. cloiselii* and *S. minutifolia*. *Secamone cloiselii* was thought to be morphologically more similar to *S. geayi* (Klackenberg, 1992a) due to a kind of small leaf hair that is unique in *Secamone*. In the present analysis, however, these two species are

placed in two different clades. *Secamone minutifolia*, with its characteristic equifacial leaves, has not been assigned to a morphological group and does not show clear similarities with the other species (Klackenberg, 1992a). Species composing clade 2 share with the two exemplars from the *S. cristata* group (clade 1) a common type of compact pollinarium (Civeyrel, unpublished). These species form a well-supported (BS = 78%; PP = 100%) clade, suggesting that the compact pollinarium with the four pollinia supported by a narrow corpuscle and a short caudicle without a distinct shape is of good phylogenetic value for this group of *Secamone*. The species of this clade are found in diverse phytogeographical areas across Madagascar.

Clade 3. This strongly supported clade (BS = 100%) includes *Secamone ligustrifolia* and *S. buxifolia*. Together with two more species not analyzed here they form the *S. ligustrifolia* morphological species group, which is characterized by large and fleshy coronal lobes with rounded back and a style-head with a distinctive lower and broader part (Klackenberg, 1992a, 2000a). *Secamone ligustrifolia* and *S. buxifolia* are distributed sympatrically in central Madagascar. They have the same type of pollinarium (Civeyrel, unpublished), with an elongated caudicle bearing four almost sessile pollinia on the back.

Clade 4. Four species, *Secamone glaberrima*, *S. uncinata*, *S. tenuifolia*, and *S. sparsiflora* form this strongly supported clade (BS = 99%). The *S. tenuifolia* group, with eight species (Klackenberg, 1992a, 2000b) distributed mostly in central but also in eastern Madagascar, is characterized by having thick corolla lobes finely puberulous on their inner side and well-developed corona lobes narrowed and puberulous in their basal part. *Secamone uncinata* from southwestern Madagascar does not share these characters, but the flowers are valvate as in the *S. tenuifolia* group, in contrast to the more general state of overlapping corolla lobes that is characteristic for all other *Secamone* included in this study, as well as for *Pervillaea* and *Secamonopsis*. Furthermore, the species in clade 4 show clear similarities in their pollinaria, which have no synorganization between pollinia, but this character is believed to be plesiomorphic (Civeyrel & Rowe, 2001).

Secamone elliotii K. Schum. is the only species out of the nine belonging to the *S. bicolor* group (Klackenberg, 1992a, 2005b) that has been included in the present analysis. This could explain its

unresolved position within the Malagasy *Secamone*. The *S. bicolor* group is characterized by entirely glabrous corollas, minute staminal columns with thin corona lobes, and tuberculate-papillate leaf epidermis. The positions of *S. falcata* Klack. and *S. grandiflora* Klack. are also unresolved. *Secamone grandiflora* belongs together with four more species to the *S. ankarensis* group of Klackenberg (1992a, 2000c). They are characterized by large flowers (ca. 1 cm long), distinctive falcate corona lobes, and a distinctly protruding and deeply bifid style-head. Morphologically, *S. falcata* adheres to this group but differs by its shorter style-head and smaller leaves on brachyblasts.

PHYLOGENETIC VALUE OF THE INDELS RELATED TO THE SECAMONOIDEAE

In the coding gene *matK*, the most informative indels (Appendix 2) are the five bp deletion (sites 81 to 85, Appendix 2) and the four bp deletion (sites 92 to 95), both found in a previous study (Civeyrel & Rowe, 2001) for the clade of Malagasy *Secamone*. These deletions are not found in *S. volubilis* distributed in La Réunion, nor in *S. oleaefolia* from central Madagascar. These two species also show an insertion of six bp in the *matK* gene from sites 75 to 80.

In the non-coding region *trnT-L*, seven indels are phylogenetically informative for the Secamonoideae. The entire Secamonoideae can be characterized by an insertion of 10 bp localized from sites 1833 to 1842 (Appendix 2). The clade including *Secamone* and *Secamonopsis* can be characterized by an insertion of seven bp from sites 1918 to 1924. The two species of the genus *Secamonopsis* can be characterized by a deletion of seven bp from sites 1782 to 1788. The clade of Malagasy *Secamone* (excluding *S. oleaefolia*) shows an insertion of seven bp from sites 1911 to 1917. Within this clade, a group including *S. cloiselii*, *S. ecoronata*, and *S. urceolata* (clade 2, Fig. 2), is characterized by an insertion of five bp from sites 1620 to 1624. The *Secamone volubilis*–*S. oleaefolia* clade shows an insertion of two bp from sites 1739 to 1740, and a deletion of one nucleotide at the position 1994.

The interestingly conservative *trnL* intron does not show unambiguous informative indels for the Secamonoideae. The *trnL-F* spacer shows two informative indels: an insertion of one nucleotide at position 3214 shared by the entire Secamonoideae, and a deletion of one nucleotide at the site 3380 shared by all analyzed species of *Pervillaea*. Each major taxonomic group within the Secamonoideae shows at least one characteristic indel. Regarding the phylogenetic value of the indels within the subfamily, most noteworthy is

the high number of indels characterizing the *Secamone volubilis*–*S. oleaefolia* clade, which are not present in the remaining Malagasy *Secamone*. However, this molecular evidence is not incongruent with the current generic boundaries in Secamonoideae. Consequently, based on the present sampling, it seems premature to redraw generic circumscriptions, as *S. volubilis* and *S. oleaefolia* both fit well in *Secamone* morphologically. On the other hand, *Secamonopsis*, which is morphologically distinct with its characteristic two-armed caudicles, is at present best kept as a separate genus.

ISLAND COLONIZATION AND DIVERSIFICATION: THE RADIATION OF THE SECAMONOIDEAE IN MADAGASCAR

Sequence divergence calculated among the Malagasy *Secamone* species ranged from 0.03% to 1.6%, and for most of the species branch lengths do not exceed 10 (Fig. 2) on a total tree length of 1153. This low sequence variation is concomitant with an important morphological diversification, namely, the morphology of staminal coronas and style-heads that characterizes different groups of *Secamone* (Klackenberg, 1992a). This low sequence divergence coupled with an important morphological differentiation (here the breeding system) is consistent with other molecular studies related to adaptive radiation of taxa in islands or other specific areas, such as high African mountains (Knox & Palmer, 1995; Böhle et al., 1996; Kim et al., 1996; Baldwin et al., 1998; Panero et al., 1999; Moore et al., 2002; Mort et al., 2002; Bruyns & Klak, 2004). The Secamonoideae have colonized all of the phytogeographical domains in Madagascar with a high percentage of endemism, with around 80% of the species endemic to one domain only (Klackenberg, 1992a). Sympatric distributions of species are not rare. For example, *S. sparsiflora* and *S. minutifolia*, belonging to two different groups within *Secamone* but very similar in habit, are both endemic to the Isalo plateau and can be found within a few meters of each other. The important radiation and speciation of the Secamonoideae observed in Madagascar could be attributed to successive geographic vicariance events across different ecological zones. To test this hypothesis, it is necessary to get a more complete phylogeny of the Malagasy *Secamone*.

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Appendix 1. Taxa used in the molecular phylogeny of Secamonoideae. Accession numbers for sequences in GenBank are provided. *matK* (maturase-encoding gene), *trnT-L* (spacer), *trnL* (intron), and *trnL-F* (spacer) are sequences in the chloroplast genome. New sequences for this study are represented by an asterisk; unavailable sequences are represented by a dash.

Taxon	Taxon voucher, herbarium, origin	GenBank accession number			
		<i>matK</i>	<i>trnT-L</i>	<i>trnL</i>	<i>trnL-F</i>
OUTGROUP					
Rauvolfioideae					
<i>Acokanthera oblongifolia</i> (Hochst.) Codd	<i>Civeyrel 1053</i> , TL, South Africa	Z70182	AY899957	DQ221123*	DQ221165*
Apocynoideae					
<i>Beaumontia grandiflora</i> Wall.	<i>Civeyrel 1071</i> , TL, India	Z98174	AY899958	DQ221124*	DQ221166*
<i>Nerium oleander</i> L.	<i>Civeyrel 1079</i> , TL, Europe	Z98173	AY899959	DQ221125*	DQ221167*
INGROUP					
Apocynoideae					
<i>Baissea zygodiioides</i> Stapf	<i>P. Endress 99-02</i> , Z, Ivory Coast	DQ221120*	—	DQ221126*	DQ221168*
<i>Motandra guineensis</i> (Thonn.) A. DC.	<i>Pascal Markin s.n.</i> , Z, Ivory Coast	DQ221121*	—	DQ221127*	DQ221169*
<i>Oncinotis tenuiloba</i> Stapf	<i>Abbot 7707</i> , Z, South Africa	DQ221122*	—	DQ221128*	DQ221170*
Asclepiadoideae					
<i>Araujia sericifera</i> Brot.	<i>Civeyrel 1059</i> , TL, South America	Z98194	AY899960	DQ221129*	DQ221171*
<i>Cynanchum acutum</i> L.	<i>Civeyrel 1644</i> , TL, France (cultivated)	AY899939	AJ428552	AJ128583	AJ428584
<i>Fockea capensis</i> Endl.	<i>Civeyrel 1067</i> , TL, South Africa	Z98187	AY899961	DQ221131*	DQ221173*
<i>Gonolobus xanthotrichus</i> Brandegees	<i>Civeyrel 1060</i> , TL, Mexico	Z98195	AY899962	DQ221133*	DQ221175*
<i>Pergularia daemia</i> Chiov.	<i>Civeyrel 1000</i> , TL, India	Z98191	AJ290891	AJ290892	AJ290893
<i>Riocreuxia burchellii</i> Schum.	<i>Civeyrel 1109</i> , TL, Africa	Z98190	AY899963	DQ221143*	DQ221185*
<i>Vincetoxicum nigrum</i> (L.) Moench	<i>Civeyrel 1106</i> , TL, Europe	Z98192	AY899964	AF214451	AF214297
Periplocoideae					
<i>Camptocarpus mauritianus</i> Decne.	<i>Civeyrel 1062</i> , TL, Réunion Island	Z98175	AY899965	DQ221130*	DQ221172*
<i>Ischnolepis graminifolia</i> (Costantin & Gallaud) Klack.	<i>Civeyrel 1246</i> , TL, Madagascar	AY899940	AY899966	DQ221134*	DQ221176*
<i>Mondia ecornuta</i> (N. E. Br.) Bullock	<i>Sennblad 215</i> , K, Africa	AY899941	AY899967	DQ221135*	DQ221177*
<i>Pentopetia grevei</i> (Baill.) Venter	<i>Civeyrel 1222</i> , TL, Madagascar	AY899943	AY899968	DQ221132*	DQ221174*
<i>Pentopetia lutea</i> Klack. & L. Civeyrel	<i>Civeyrel, 1243b</i> , TL, Madagascar	AY899944	AY899969	DQ221136*	DQ221178*
<i>Periploca graeca</i> L.	<i>Civeyrel 1083</i> , TL, Greece	Z98178	AY899970	AF102468	AF214244
<i>Petopentia natalensis</i> (Schltr.) Bullock	<i>Civeyrel 1173</i> , TL, France (cultivated)	AY903453	AJ581810	DQ221141*	DQ221183*
<i>Raphionacme welwitschii</i> Schltr. & Rendle	<i>Civeyrel 1088</i> , TL, Zaire	Z98179	AY899971	DQ221142*	DQ221184*
<i>Tacazzea apiculata</i> Oliv.	<i>Venter 9188</i> , K, Africa	AY899945	AY899972	DQ221164*	DQ221206*

Appendix 1. Continued.

Taxon	Taxon voucher, herbarium, origin	GenBank accession number			
		<i>matK</i>	<i>trnT-L</i>	<i>trnL</i>	<i>trnL-F</i>
Secamonoideae					
<i>Pervillaea decaryi</i> (Choux) Klack.	<i>Civeyrel 3471</i> , TL, Madagascar	AY899946	AY899973	DQ221137*	DQ221179*
<i>Pervillaea phillipsonii</i> Klack.	<i>Civeyrel 1241</i> , TL, Madagascar	AJ312408	AY899976	DQ221140*	DQ221182*
<i>Pervillaea tomentosa</i> Decne.	<i>Civeyrel 1522</i> , TL, Madagascar	AY899947	AY899975	DQ221139*	DQ221181*
<i>Pervillaea venenata</i> (Baill.) Klack.	<i>Civeyrel 1248</i> , TL, Madagascar	Z98181	AY899974	DQ221138*	DQ221180*
<i>Secamone bosseri</i> Klack	<i>Civeyrel 1267</i> , TL, Madagascar	Z98182	AY899977	DQ221144*	DQ221186*
<i>Secamone buxifolia</i> Decne.	<i>Civeyrel 1322</i> , TL, Madagascar	AJ312405	AY899978	DQ221145*	DQ221187*
<i>Secamone castanea</i> Klack.	<i>Civeyrel 1356</i> , TL, Madagascar	AY899948	AY899979	DQ221146*	DQ221188*
<i>Secamone cloiselii</i> Choux	<i>Civeyrel 1312</i> , TL, Madagascar	AY899949	AY899980	DQ221147*	DQ221189*
<i>Secamone cristata</i> Jum. & H. Perrier	<i>Civeyrel 1320</i> , TL, Madagascar	AJ312400	AY899981	DQ221148*	DQ221190*
<i>Secamone ecoronata</i> Klack.	<i>Civeyrel 1261</i> , TL, Madagascar	AJ312407	AY899982	DQ221149*	DQ221191*
<i>Secamone elliottii</i> K. Schum.	<i>Civeyrel 1304</i> , TL, Madagascar	AJ312402	AY899983	DQ221150*	DQ221192*
<i>Secamone falcata</i> Klack.	<i>Civeyrel 1228</i> , TL, Madagascar	AJ312404	AY899984	DQ221151*	DQ221193*
<i>Secamone geayi</i> Costantin & Gallaud	<i>Civeyrel 1200</i> , TL, Madagascar	Z98184	AY899985	DQ221152*	DQ221194*
<i>Secamone glaberrima</i> K. Schum.	<i>Civeyrel 1335</i> , TL, Madagascar	AY899950	AY899986	AF214420	AF214266
<i>Secamone grandiflora</i> Klack.	<i>Civeyrel 1292</i> , TL, Madagascar	AY899951	AY899987	DQ221153*	DQ221195*
<i>Secamone humbertii</i> Choux	<i>Civeyrel 1293</i> , TL, Madagascar	AY899952	AY899988	DQ221154*	DQ221196*
<i>Secamone ligustrifolia</i> Decne.	<i>Civeyrel 1323b</i> , TL, Madagascar	AY899953	AY899989	DQ221155*	DQ221197*
<i>Secamone minutifolia</i> Choux	<i>Civeyrel 1257</i> , TL, Madagascar	AJ312406	AY899990	DQ221156*	DQ221198*
<i>Secamone oleaefolia</i> Decne.	<i>Civeyrel 1770</i> , TL, Madagascar	AY899954	AY899991	AF214421	AF214267
<i>Secamone sparsiflora</i> Klack.	<i>Civeyrel 1244</i> , TL, Madagascar	AJ312401	AY899992	DQ221157*	DQ221199*
<i>Secamone tenuifolia</i> Decne.	<i>Civeyrel 1259</i> , TL, Madagascar	AY899955	AY899993	DQ221158*	DQ221200*
<i>Secamone uncinata</i> Choux	<i>Civeyrel 1309</i> , TL, Madagascar	AJ312403	AY899994	DQ221160*	DQ221202*
<i>Secamone urceolata</i> Klack.	<i>Civeyrel 1299</i> , TL, Madagascar	AY899956	AY899995	DQ221159*	DQ221201*
<i>Secamone volubilis</i> (Lam.) Marais	<i>Civeyrel 1092</i> , TL, Réunion Island	Z98186	AY899996	DQ221161*	DQ221203*
<i>Secamonopsis madagascariensis</i> Jum.	<i>Civeyrel 1262</i> , TL, Madagascar	Z98185	AY899998	DQ221163*	DQ221205*
<i>Secamonopsis microphylla</i> L. Civeyrel & Klack.	<i>Civeyrel 1206</i> , TL, Madagascar	AJ312409	AY899997	DQ221162*	DQ221204*

Appendix 2. Informative indels related to the Secamonoideae. ? represents base not available.

			<i>trnT-L</i>						<i>trnL-F</i>	
<i>MatK</i>			11111111	1111	1111111111	111111111111	1111111111111111	111	3	3
			6666666	7777	777777777	8888888888	9999999999999999	999	2	3
777777888888		9999	1222222	3344	888888888	33333333444	1111111111222222	999	1	8
456789012345		2345	9012345	8901	123456789	23456789012	0123456789012345	345	4	0
<i>Acokanthera oblongifolia</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	G-----	T-----A	TAA	-	T
<i>Beaumontia grandiflora</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATAAT---	T-----C	TAA	-	T
<i>Verium oleander</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	G-----	T-----C	TAA	-	T
<i>Baissea zygodioides</i>	T-----AGTTT	AAAC	???????	????	?????????	???????????	???????????	???	-	T
<i>Motandra guineensis</i>	T-----AGTTT	AAAC	???????	????	?????????	???????????	???????????	???	-	T
<i>Oncinotis tenuiloba</i>	T-----AGTTT	AAAC	???????	????	?????????	???????????	???????????	???	-	T
<i>Araujia sericifera</i>	T-----AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G-----	T-----C	TAA	-	T
<i>Cynanchum acutum</i>	T-----AGTTT	AAAC	A-----T	----	-----	-----	-----	TAA	-	T
<i>Fockea capensis</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	G-----	T-----C	TAA	-	T
<i>Gonolobus xanthotrichus</i>	T-----AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G-----	T-----C	TAA	-	G
<i>Pergularia daemia</i>	T-----AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G-----	T-----C	TAA	-	T
<i>Riocreuxia burchellii</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	G-----	T-----C	TAA	-	T
<i>Vincetoxicum nigrum</i>	T-----AGTTT	AAAC	A-----T	A--G	TAGAATTTA	G-----	T-----C	TAA	-	T
<i>Camptocarpus mauritanus</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	T
<i>Ischnolepis graminifolia</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	T
<i>Mondia ecornuta</i>	T-----AGTTT	AAAC	A-----A	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	T
<i>Pentopetia grevel</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	T
<i>Pentopetia lutea</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	?
<i>Periploca graeca</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTC	GATATCTTATA	T-----C	TAA	-	T
<i>Petopentia natalensis</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	T
<i>Raphionacme welwitschii</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	T
<i>Tacazzea apiculata</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATCTT---	T-----C	TAA	-	?
<i>Perrillaea decaryi</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATTTATTACG	T-----C	TAA	T	-
<i>Perrillaea venenata</i>	T-----AGTTT	AAAC	A-----T	A--A	TACAATTTA	GATTTATTACG	T-----C	TAA	T	-
<i>Perrillaea tomentosa</i>	T-----AGTTT	AAAC	A-----T	A--G	TACAATTTA	GATATATTACG	T-----C	TAA	T	-
<i>Perrillaea phillipsonii</i>	T-----AGTTT	AAAC	A-----T	A--A	TACAATTTA	GATTTATTACG	T-----C	TAA	T	-
<i>Secamone bosseri</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone buxifolia</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone castanea</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone cloiselii</i>	T-----	----	ATGTTAT	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T

Appendix 2. Continued.

			<i>trnT-L</i>						<i>trnL-F</i>	
<i>MatK</i>			11111111	1111	111111111	11111111111	1111111111111111	111	3	3
			6666666	7777	777777777	88888888888	9999999999999999	999	2	3
777777888888	9999		1222222	3344	888888888	33333333444	1111111111222222	999	1	8
456789012345	2345		9012345	8901	123456789	23456789012	0123456789012345	345	4	0
<i>Secamone cristata</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone ecoronata</i>	T-----	----	ATGTTAT	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone elliottii</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone falcata</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone geayi</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATTTTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone glaberrima</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	T????????????????	???	T	T
<i>Secamone grandiflora</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone humbertii</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone ligustrifolia</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone minutifolia</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone sparsiflora</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone tenuifolia</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone urceolata</i>	T-----	----	ATGTTAT	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone uncinata</i>	T-----	----	A-----T	A--G	TACAATTTA	GATATATTACG	TTTAGATTTCGAGATTC	TAA	T	T
<i>Secamone oleaefolia</i>	TGACCATGGTTT	AAAC	A-----T	AGCG	TACAATTTA	GATATATTACG	T-----CGAGATTC	T-A	T	T
<i>Secamone volubilis</i>	TGACCATGGTTT	AAAC	A-----T	AGCG	TACAATTTA	GATATATTACG	T-----CGAGATTC	T-A	T	T
<i>Secamonopsis microphylla</i>	T-----GGTTT	AAAC	A-----T	A--G	T-----A	GATATATTACG	T-----CGAGATTC	TAA	T	G
<i>Secamonopsis</i> <i> madagascariensis</i>	T-----GGTTT	AAAC	A-----T	A--G	T-----A	GATATATTACG	T-----CGAGATTC	TAA	T	T